

**Figure 1: Novel Gene Sequence Analysis**

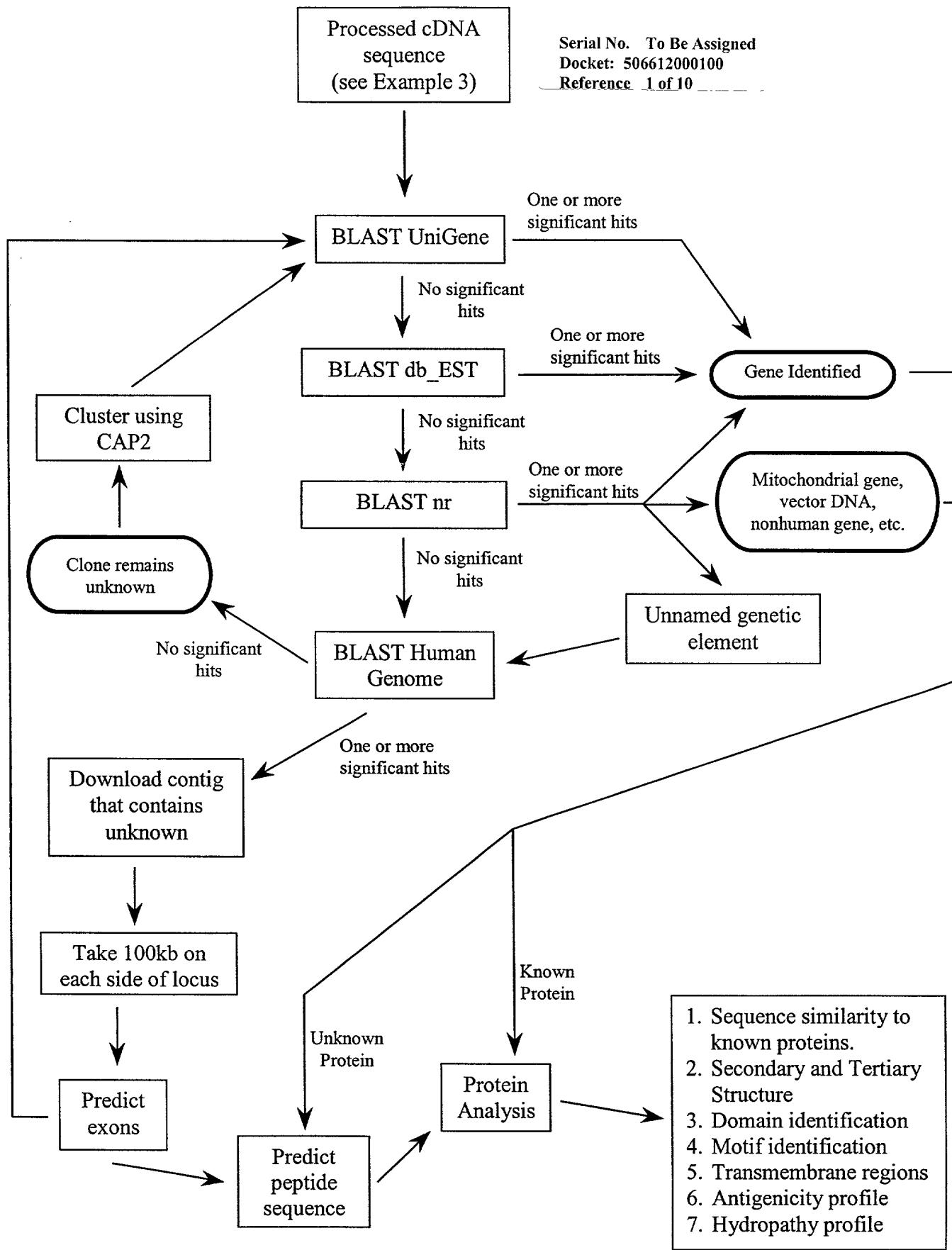
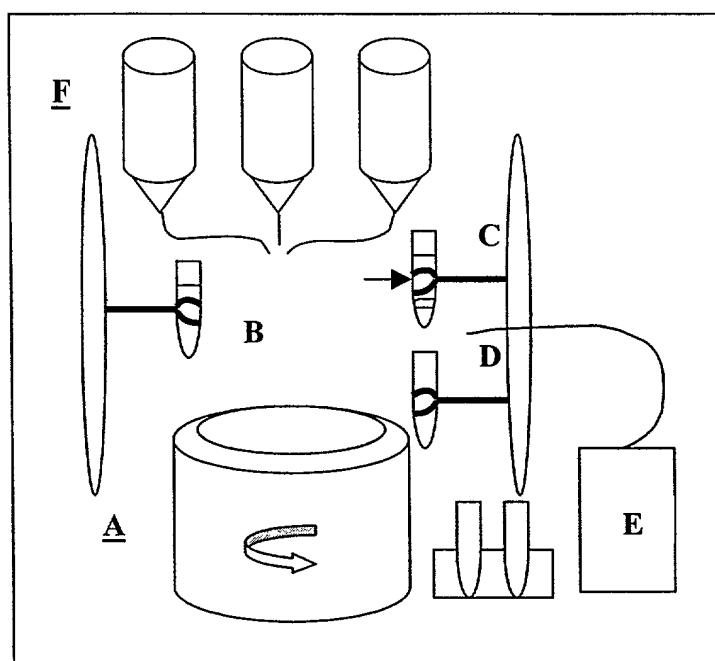


Figure 2 . Automated Mononuclear Cell RNA Isolation Device



**Figure 3: Kits for discovery of, or application of diagnostic gene sets**

**A. Contents of kit for discovery of diagnostic gene sets**

1. Sterile, endotoxin and RNase free blood collection tubes (>10cc capacity)
2. Alcohol swabs, tourniquet, 18g needle and syringe (>10cc capacity)
3. Erythrocyte lysis buffer
4. Leukocyte lysis buffer
5. Substrates for labeling of RNA (may vary for various expression profiling techniques)  
For fluorescence cDNA microarray expression profiling:  
Reverse transcriptase and 10x RT buffer  
Poly-dT primer  
DTT  
Deoxynucleotides 100mM each  
RNase inhibitor  
Cy3 and Cy5 labeled deoxynucleotides
6. cDNA microarrays containing candidate gene libraries
7. Cover slips for slides
8. hybridization chambers
9. Software package for identification of diagnostic gene set from data  
Contains statistical methods.  
Allows alteration in desired sensitivity and specificity of gene set.  
Software facilitates access to and data analysis by centrally located database server.
10. Password and account number to access central database server.
11. Kit User Manual

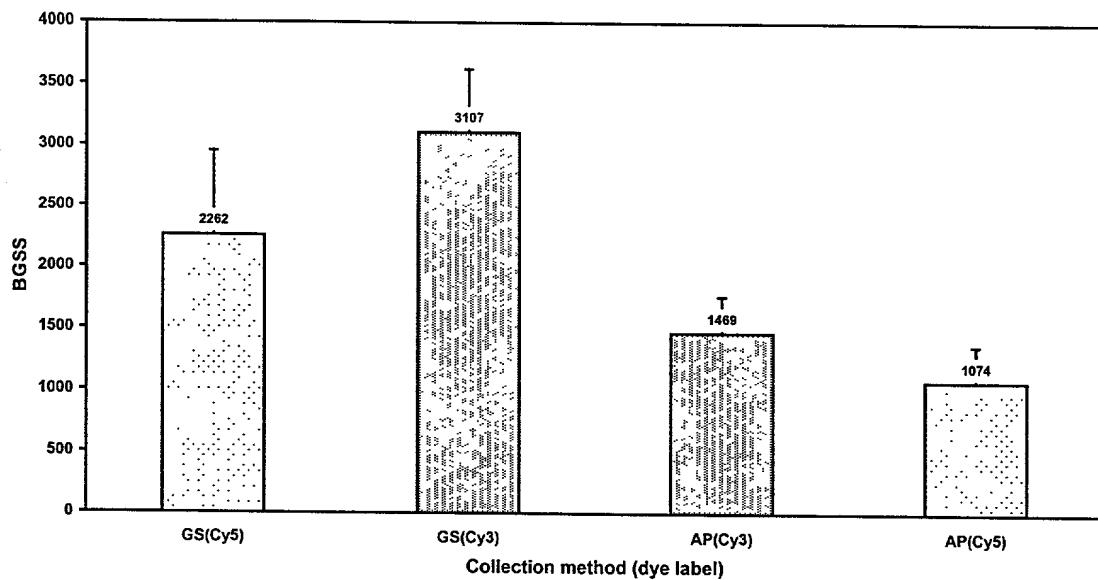
**B. Contents of kit for application of diagnostic gene sets**

1. Sterile, endotoxin and RNase free blood collection tubes (>10cc capacity)
2. Alcohol swabs, tourniquet, 18g needle and syringe (>10cc capacity)
3. Erythrocyte lysis buffer
4. Leukocyte lysis buffer
5. Substrates for labeling of RNA (may vary for various expression profiling techniques)  
For fluorescence cDNA microarray expression profiling:  
Reverse transcriptase and 10x RT buffer  
Poly-dT primer  
DTT  
Deoxynucleotides 100mM each  
RNase inhibitor  
Cy3 and Cy5 labeled deoxynucleotides
6. cDNA microarrays containing diagnostic gene sets
7. cover slips for slides
8. hybridization chambers
9. Software package for identification of diagnostic gene set from data  
Contains statistical methods.  
Allows alteration in desired sensitivity and specificity of gene set.  
Software facilitates access to and data analysis by centrally located database server
10. Password and account number to access central database server.
11. Kit User Manual

**Figure 4**

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**Comparison of Guanine-Silica (GS) to  
Acid-Phenol (AP) RNA Purification**



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Expression of Leukocyte Specific Genes

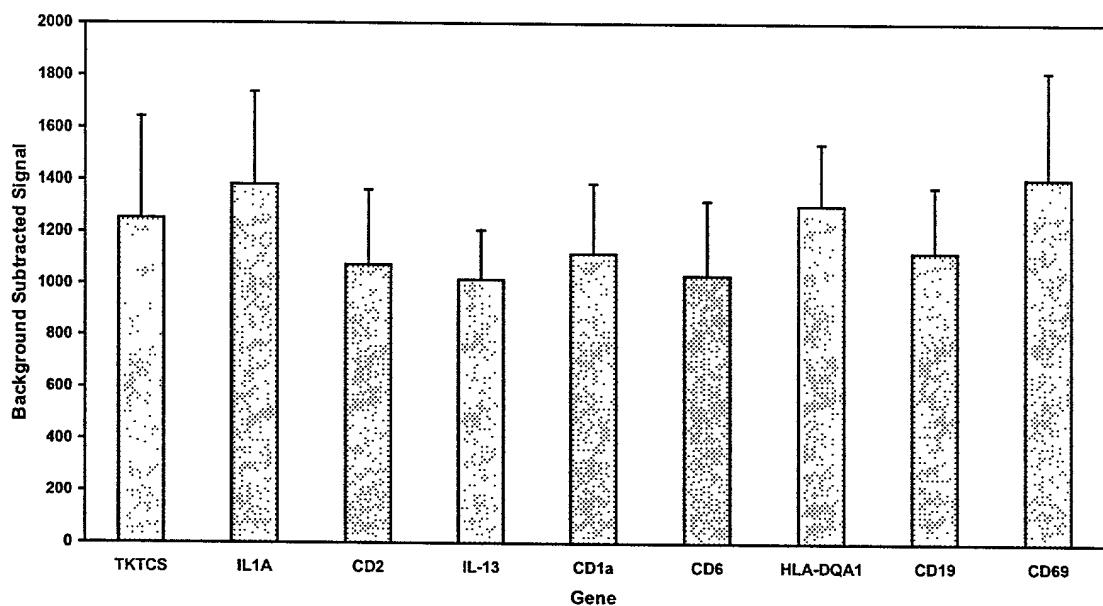
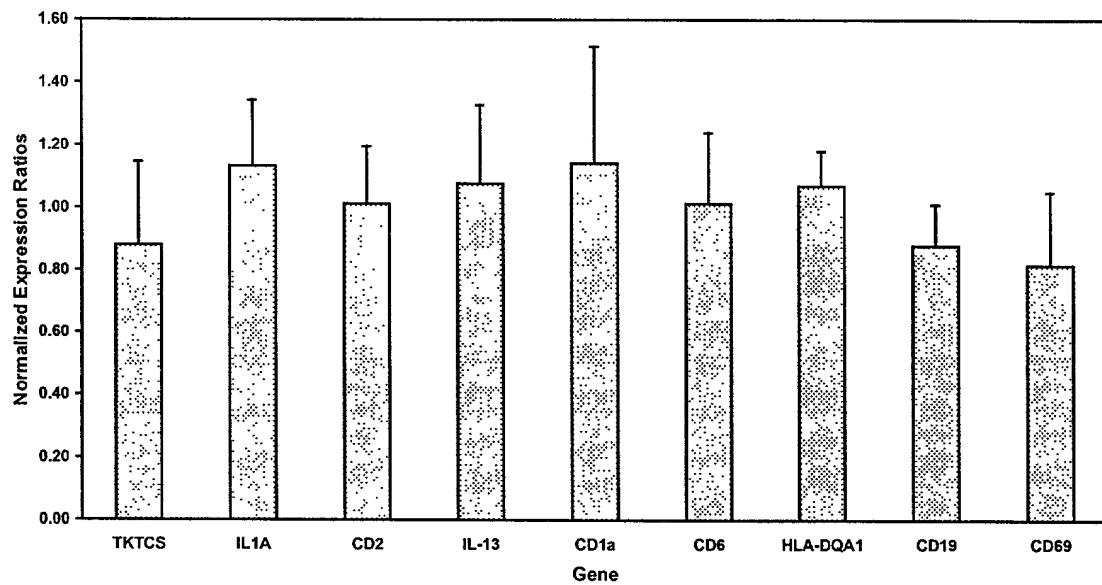


Figure 5

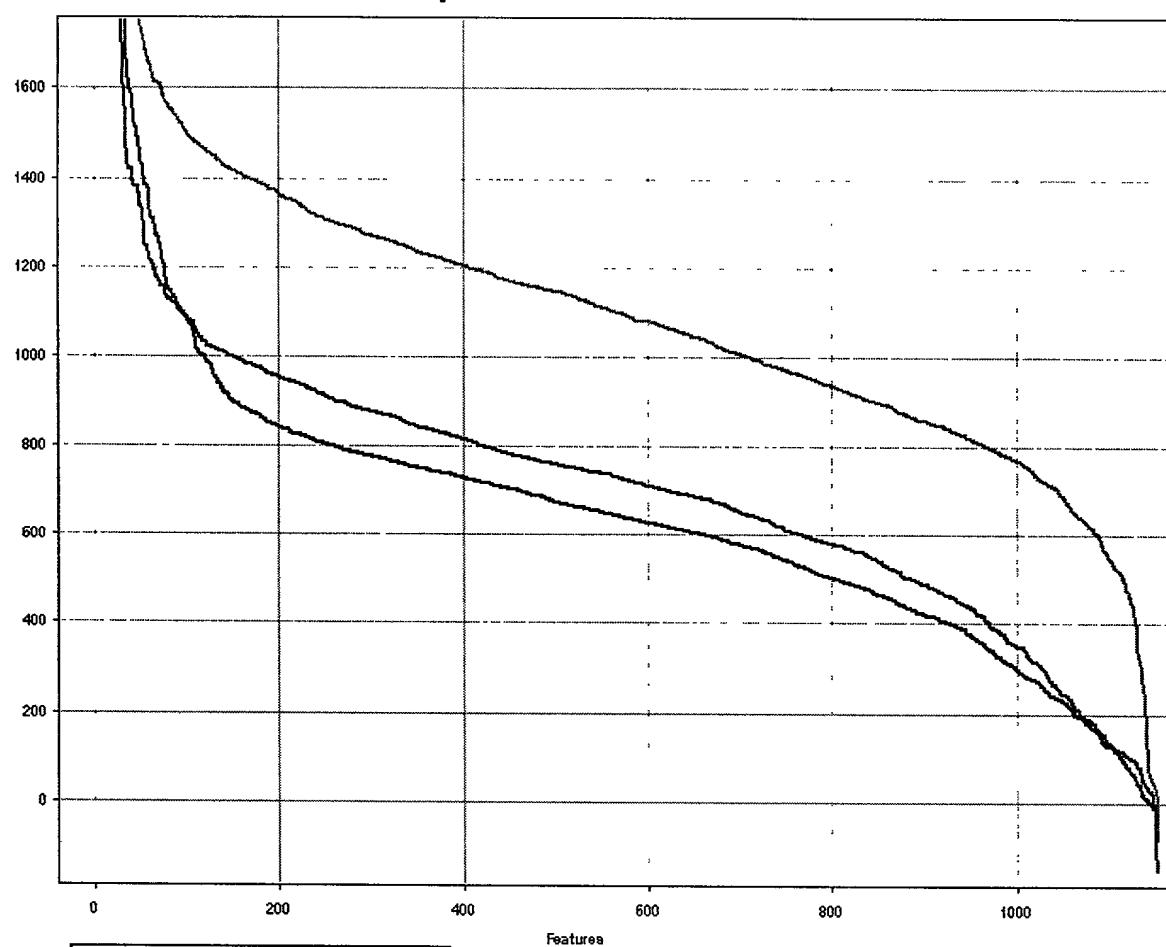
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**Figure 6**

**Expression of Leukocyte-Specific Genes**



### Comparison of Control RNAs



All columns use the same scale.

- Mononuclear cells, resting and stimulated
- 10 Buffy Coats, resting
- Mononuclear cells, resting

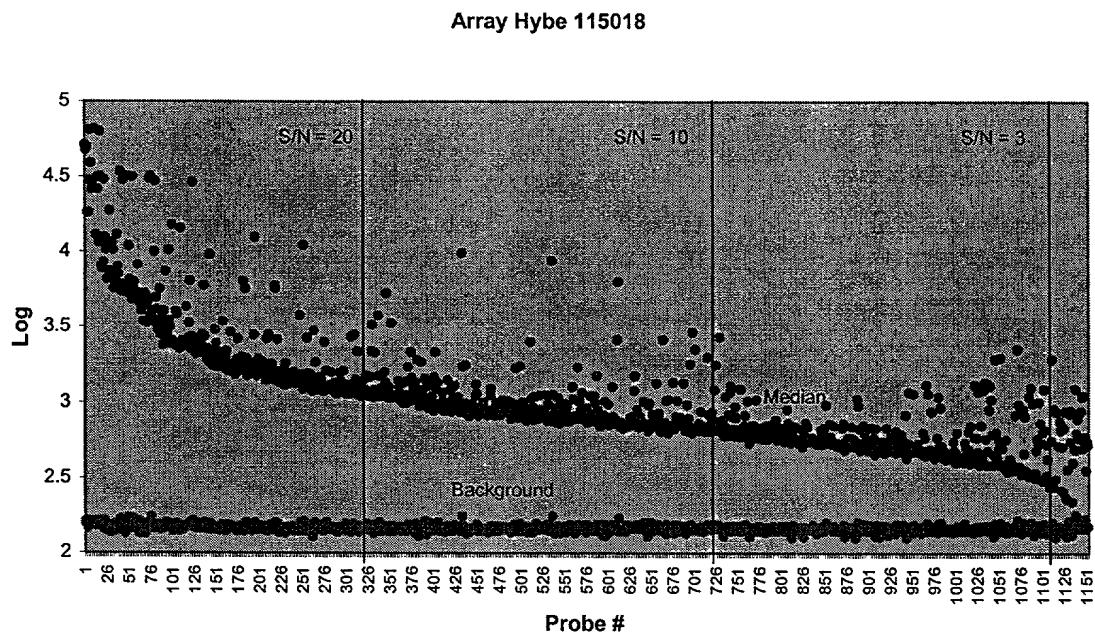
All markers are connected and ordered by Features.

10 µg of each control RNA was labeled.

Figure 7

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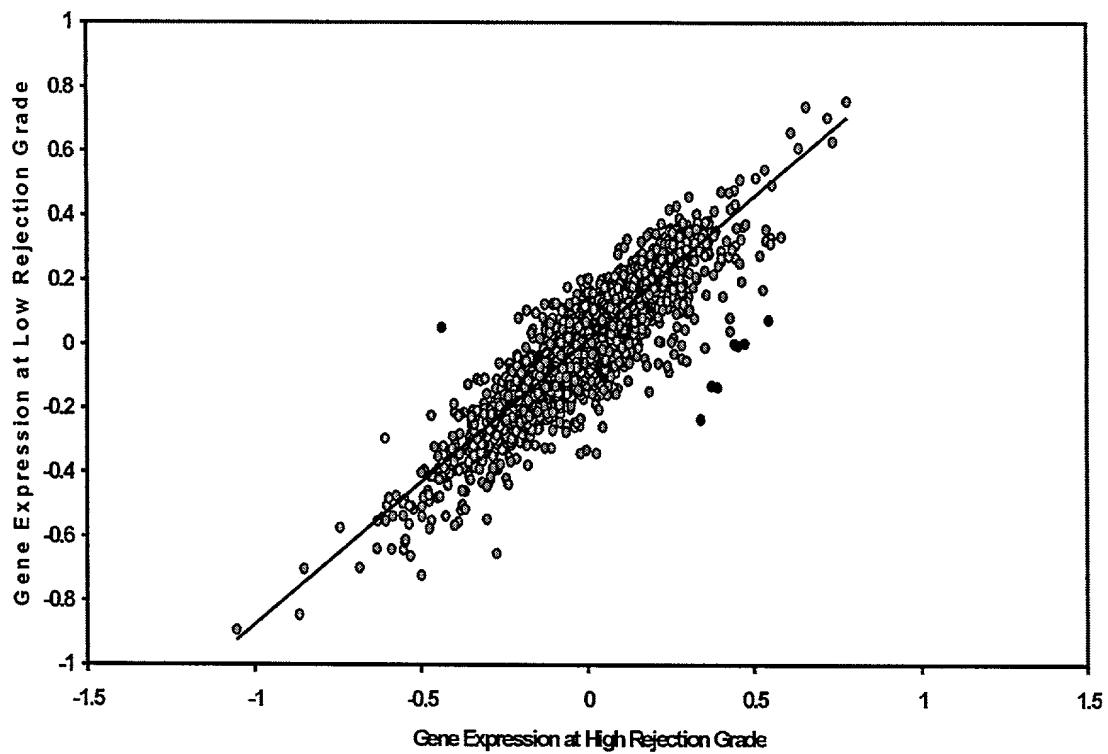
**Figure 8:** Log expression of each probe using the R50 reference RNA. Probe expression is ordered by Signal to noise, S/N, decreasing from left to right.



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Figure 9

Comparison of High Rejection Grade to Low Rejection Grade



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Figure 10: Differential gene expression between grade 0 and 3A samples:

Probe		Array 107742: Grade 0						Array 107739: Grade 3A						Ratio of SRs	
Acc #	Name	Oligo ID	F633	F532	SR:	F633	F532	SR:	F633	F532	SR:	Grade 0/3A	Grade 3A/0		
			Median - B633	Median - B532	Cy3/Cy5 ratio (g/r)		Median - B532	Median - B633		Median - B633	Median - B532	Cy3/Cy5 ratio (g/r)			
NM_003202	transcription factor 7 (T-cell specific, HMG-box) (TCF7), mRNA	2476	5558	1050	0.188917	0.710038	5827	358	0.061438	0.219793	3.23048873	0.30955069			
BE220959	major histocompatibility complex, class II, DQ beta 1 (HLA-DQ $\beta$ 1), mRNA	6025	1810	635	0.350829	1.318579	2150	252	0.117209	0.419312	3.14462275	0.31800317			
BE220959	major histocompatibility complex, class II, DQ beta 1 (HLA-DQ $\beta$ 1), mRNA	6025	1402	487	0.347361	1.305545	2121	247	0.116455	0.416612	3.13371968	0.31910959			
NM_002922	regulator of G-protein signalling 1 (RGS1), mRNA	2407	804	95	0.118159	0.444098	1884	75	0.039809	0.142415	3.11833431	0.32068403			
NM_001781	CD69 antigen (p60, early T-cell activation antigen) (CD6)	2192	4121	405	0.098277	0.369371	7385	254	0.034394	0.123043	3.00195843	0.33311587			
NM_002341	lymphotoxin beta (TNF superfamily, member 3) (LTB), mRNA	2283	13488	3447	0.255556	0.960516	29882	2727	0.091259	0.326476	2.94207495	0.33989617			
BE220959	major histocompatibility complex, class II, DQ beta 1 (HLA-DQ $\beta$ 1), mRNA	6025	1539	515	0.334633	1.257707	1942	237	0.122039	0.436591	2.88074602	0.3471323			
NM_001781	CD69 antigen (p60, early T-cell activation antigen) (CD6)	2192	3850	386	0.10026	0.376823	7705	282	0.0366	0.130934	2.87796556	0.34746767			
U05040	far upstream element (FUSE) binding protein 1 (FUBP1), mRNA	3581	4507	1119	0.24828	0.933154	2390	220	0.09205	0.329306	2.83369583	0.35289603			
X14908	nuclear receptor subfamily 4, group A, member 2 (NR4A2), mRNA	3729	1365	167	0.122344	0.459827	9541	434	0.045488	0.162731	2.82568319	0.35389672			
NM_003202	transcription factor 7 (T-cell specific, HMG-box) (TCF7), mRNA	2476	2716	486	0.17894	0.672539	5310	356	0.067043	0.239845	2.80405488	0.3566264			
AF035947	cytokine-inducible inhibitor of signalling type 1b mRNA	642	9850	5254	0.533401	2.004771	969	197	0.203302	0.727307	2.75642938	0.36278818			
NM_001781	CD69 antigen (p60, early T-cell activation antigen) (CD6)	2192	3357	356	0.106047	0.398574	5963	246	0.041254	0.147586	2.70062225	0.37028503			
Y14737	mRNA for immunoglobulin lambda heavy chain /cds=(65	4905	1390	248	0.178417	0.670576	6561	5767	0.878982	3.144527	0.21325167	4.68929496			
Y14737	mRNA for immunoglobulin lambda heavy chain /cds=(65	4905	1398	240	0.171674	0.645231	7159	6112	0.853751	3.054262	0.21125576	4.73359863			
BC006402	mRNA for immunoglobulin lambda heavy chain /cds=(65	4481	1826	295	0.161555	0.6072	2973	2498	0.840229	3.005889	0.20200364	4.95040579			
X57812	rearranged immunoglobulin lambda light chain mRNA /c	3761	6512	747	0.114711	0.431139	27381	17730	0.647529	2.316513	0.18611538	5.37301111			
X57812	rearranged immunoglobulin lambda light chain mRNA /c	3761	6728	755	0.112218	0.421766	28820	18636	0.646634	2.313311	0.18232143	5.48481867			
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3790	8572	1188	0.138591	0.520889	17322	13892	0.801986	2.869076	0.18155283	5.50803866			
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3790	15538	2128	0.136955	0.514739	17637	14245	0.807677	2.889436	0.17814525	5.61339689			
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3791	11974	1558	0.130115	0.489034	24261	18761	0.773299	2.766449	0.17677319	5.65696646			
X57812	rearranged immunoglobulin lambda light chain mRNA /c	3761	6953	778	0.111894	0.420551	27621	18560	0.671952	2.403886	0.17494641	5.71604612			
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3791	10805	1411	0.130588	0.49081	17533	14334	0.817544	2.924735	0.16781337	5.95900079			
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3790	11246	1453	0.129201	0.4856	17074	13863	0.811936	2.904673	0.16717875	5.9816215			
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4399	2654	243	0.09156	0.344125	37518	21610	0.57599	2.060585	0.16700357	5.98789603			
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	3791	10909	1370	0.125584	0.472005	21668	18561	0.856609	3.064488	0.15402406	6.4924922			
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4399	1959	181	0.092394	0.34726	30274	19369	0.63979	2.288826	0.15171979	6.59109804			
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4399	2558	215	0.08405	0.315899	36161	21936	0.60662	2.170163	0.14556481	6.86979225			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4474	7538	684	0.09074	0.341044	6038	4037	0.668599	2.391889	0.14258368	7.01342553			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4474	8662	780	0.090048	0.338444	4339	2975	0.685642	2.45286	0.13797951	7.24745312			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4474	7183	608	0.084644	0.318133	5521	3909	0.708024	2.532931	0.12559874	7.96186351			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4475	8986	851	0.094703	0.355938	1587	1275	0.803403	2.874145	0.12384126	8.0748531			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4476	11118	1023	0.092013	0.345828	871	682	0.783008	2.801184	0.12345771	8.09993947			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4475	7428	730	0.098277	0.36937	1049	890	0.848427	3.035218	0.12169477	8.21272973			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	4476	10413	933	0.0896	0.336757	625	486	0.7776	2.781837	0.12105563	8.2606647			
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4398	5841	484	0.082863	0.311436	1694	1344	0.793388	2.838319	0.10972555	9.11364747			
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4398	7960	645	0.08103	0.304549	22985	18694	0.813313	2.909599	0.10467052	9.55378803			
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4398	11959	992	0.08295	0.311765	14170	12597	0.888991	3.180333	0.0980291	10.2010527			
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4398	6161	447	0.072553	0.272689	16180	14148	0.874413	3.128181	0.08717165	11.4716196			